

SUPER OPTIMAL AND THERMAL DEATH
TEMPERATURES OF THE COTTON PLANT AS
AFFECTED BY VARIATIONS IN RELATIVE HUMIDITY¹

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HISTORICAL REVIEW

In 1863 Sachs reported the results of an attempt to determine the effects of high temperatures on the sensitivity of *Mimosa pudica*. Transitory insensitivity, he found, was caused by an exposure of one hour to a temperature of 40° C., and at 45° C. for a half hour and 49° C. for a very brief time the same effect was produced. When permanent insensitivity was attained, at higher temperatures, death invariably followed. Sachs also reported ('64) on the effects of high temperatures on tobacco, pumpkin, corn, nasturtium, and rape, exposed for various periods of time. All the plants were able to withstand temperatures of 49–51° C., but none survived 51° C. for more than 10 minutes without injury. The power of resistance to high temperatures was found to vary at different ages. Developing leaves, stems, and roots were more easily killed than older ones.

Ewart ('03) noted a decrease in the rate of protoplasmic streaming in *Elodea*, *Tradescantia*, *Chara*, *Spirogyra*, root hairs, pollen tubes, etc., depending upon the height of the temperature above the optimum and upon the length of exposure.

Pfeffer ('03) made the generalization that all turgid plants ultimately die when the temperature reaches from 1° to 2° C. above the maximum where the plant will grow indefinitely, although growth may continue for a time, and that at temperatures of 10° C. above this maximum all flowering plants seem to be rapidly killed. He noted that plants, which at first

¹ Portions of this work, together with certain microscopical studies of the treated plants, were submitted by Mrs. Dorothy Megowen Berkley as a thesis in partial fulfillment of the requirements for the degree of master of science in the Henry Shaw School of Botany of Washington University.

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appeared fresh and unharmed after a short exposure to fatally high temperatures, frequently died later as an after-effect, even under the best external conditions.

Fung ('11) emphasized the necessity of considering the relative humidity in determining the effects of high temperature on plants. He found the maximum temperature for growth of cotton to be 113° F. in a relative humidity of 90 per cent and the optimum 85–90° F. in a relative humidity of 70.6–72.2 per cent. Cotton plants treated for four hours in a saturated atmosphere at a variety of high temperatures also gave interesting results. At 42–45° C. the stems and leaves were badly wilted but recovered; at 44–48° C. the stems and leaves were browned but new leaves appeared after one week (probably secondary growth from uninjured nodes); at 49–55° C. the plants were killed. The degree of injury was determined after the plants had been transferred to "proper conditions," which were not described. The value of Fung's results is limited by the fact that he worked with an insignificant number of plants and used only very young seedlings. Bose ('13), who gave 60° C. as the average fatal temperature for plants in general, found the death point to be lower in young plants, which confirmed the earlier statement of Sachs.

Collander ('24) determined the temperatures at which death occurred in individual cells of various plants. *Tradescantia discolor* was killed at 65° C. within an average of 1.8 minutes, *Brassica oleracea* at 60° C. within an average of 0.8 minute, *Beta vulgaris* at 60° C. in 0.7 minute, *Draparnaldia glomerata* at 55° C. in 0.32 minute, and *Pisum sativum* at 55° C. in 0.095 minute. He found that these plants could live at slightly lower temperatures for some time, thus demonstrating that the thermal death point is suddenly reached. Lepeschkin ('25) discussed the effects of optimum, maximum, and thermal death temperatures on bacteria, fungi, and higher plants. He stated that most plants died at 60–70° C. in one minute, although some died at 40–45° C. in that time.

Gilbert ('26) found that cotton grew better at 80° F. in a relative humidity of 50 per cent than it did in a relative humidity of 85 per cent. Wallace ('31) tested the effect of 1–24-hour

exposures to temperatures ranging from 15 to 60° C. on the sensitivity of *Mimosa pudica*. Above 45° C. injury or death resulted, depending upon the length of the exposure. He reported that the relative humidity had little or no effect upon the sensitivity of the plant.

Baker ('29), in studying the effects of excessively high temperatures on conifer seedlings 1-3 months old, also emphasized the suddenness at which the thermal death point was reached. The living tissues were quickly killed at 54° C. but were uninjured after a prolonged exposure to a temperature only a few degrees lower. Just below the thermal death point he noted a region of no growth where photosynthesis was apparently unable to keep up with catabolic changes, the chlorophyll decomposing faster than it was made and the leaves becoming yellow or withered. On prolonged exposure the plants died. He also found the age of the plant to be a factor in resistance, due to the development of protective tissues as the plant grew older. The indications were that there was no increased protoplasmic resistance with age.

APPARATUS

Four glass cases of identical design and size (60 in. long by 32 in. wide by 40 in. high) were used for the experimental work. Three of these cases were variously used for growing plants, for germinating seeds, and for plants under observation following treatment. Additional plants were grown on benches in the experimental room.

The fourth case was converted into the electrically controlled temperature chamber (fig. 1). The experimental plants were placed in a flat of sand which rested on a lattice rack supported 12 inches above the bottom of the case. The heating units consisted of three heaters, two of which were controlled by ordinary switches, and were placed on the floor of the case, one in either half about midway between the center line and the end. They were composed of resistance wires wrapped on racks which extended practically the width of the case. With these two heaters there were three possible combinations: when the two were in parallel maximum heat was obtained, when one was used alone approximately one-half of the maximum could be had, and

when the two were in series one-fourth of the maximum. A further reduction in temperature was obtained by turning off these heaters altogether, leaving only the third heater which was composed of resistance wire wrapped on the guard-wires of an electric fan. This heater was controlled by a Thyatron¹ and relay combination which in turn was controlled by a thermostat with a mercury make-and-break contact.

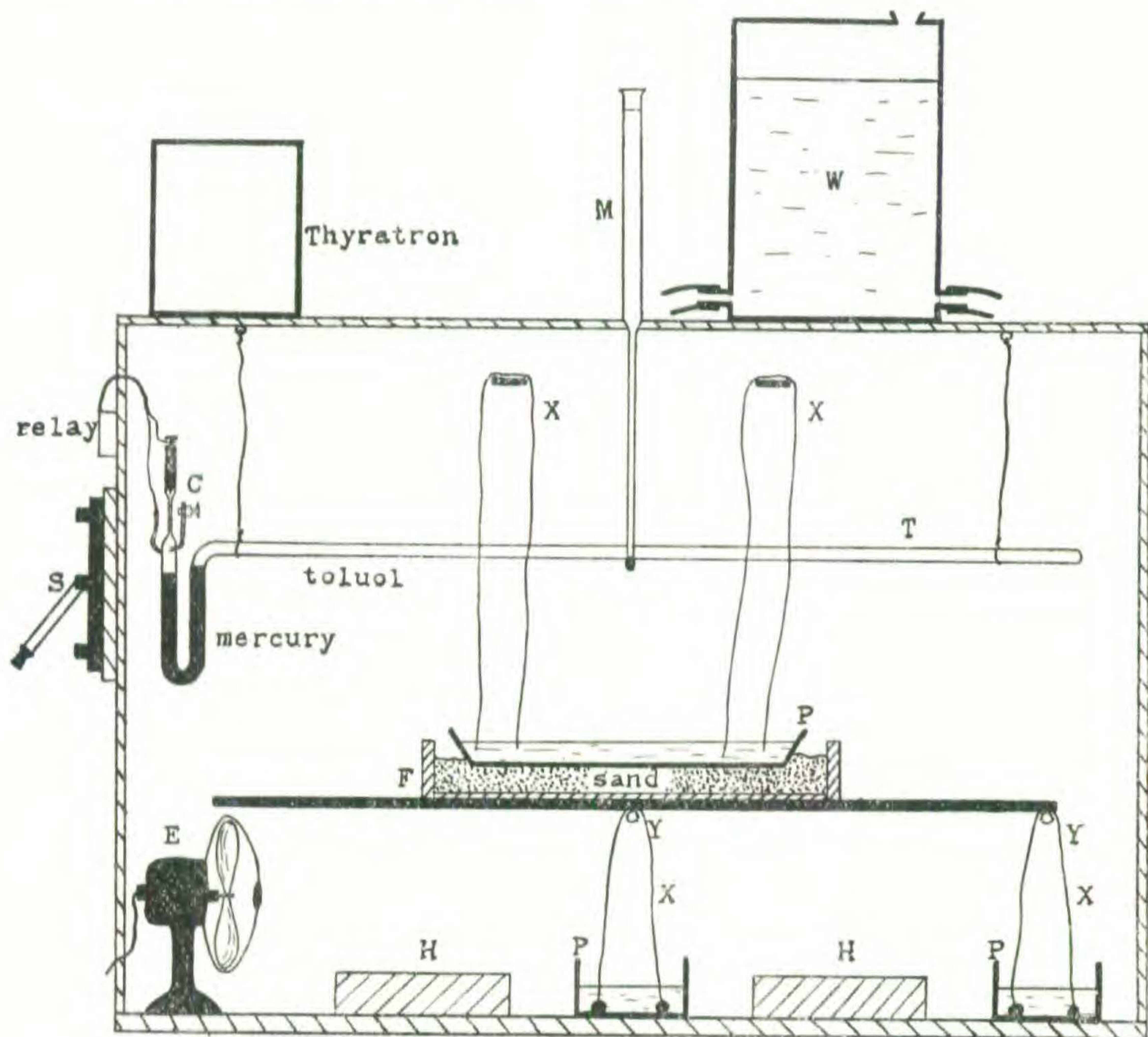


Fig. 1. Diagrammatic cross-section through the center of the controlled temperature chamber in which the plants were treated. C, capillary; E, electric fan heater; F, greenhouse flat; H, floor heaters; M, thermometer; P, water pans; S, switches; T, thermostat; W, water tank; X, humidifying cloths; Y, spray tubes.

The fan, which was located on the floor at one end of the chamber, was hooked directly to the current so that it ran continuously, blowing the warm air over the heaters and through

¹ Schmitt, F. O., and Schmitt, O. H. A. A vacuum tube method of temperature control. *Science N. S.* 73: 289-290. 1931.

humidifying cloths made of cheese-cloth. In this way the heat and moisture were uniformly distributed throughout the compartment. Good ventilation was assured by boring small holes in the frame of the compartment on either side of and in back of the fan.

The thermostat consisted of a glass tube extending the full length of the chamber, one end of which was shaped into a U terminating in a capillary. The main body of the tube had a capacity of about 200 cc. and was filled with toluol; the U tube, with a capacity of about 20 cc., contained mercury. The contact was made in the capillary in the usual manner. Due to the large capacity of this tube and the fact that it extended across the case at a level with the tops of the plants, it was possible to control the temperature within 0.5 of a degree.

The temperature of the chamber was determined by means of an incubator thermometer inserted through the top of the case, with its bulb reaching down to the level of the plants in the center.

For experiments run at high humidity, a practically saturated atmosphere was obtained by hanging from four pans at the top of the chamber strips of cloth which were kept supplied with water by tubes running from a tank above. These cloths drained into three pans below, one of which was placed in the flat of moistened sand and the others located on the floor. Additional cloths, through which the warm air from the fan was blown, extended the entire width of the temperature chamber below the rack and were moistened by constant sprays of water. An overflow in the bottom pans, which received most of the runoff water, was avoided by the use of constant-level siphons which carried off the surplus through drains in the floor.

Still other cloths were hung above either end of the pan in which the plants were placed, so that the pots of plants were not only standing in water but were more or less surrounded by moist cloths. Under these conditions the relative humidity was so high that the entire inner surface of the temperature chamber was covered with a film of water which dripped from the top and ran down the glass sides in streams.

For the low-moisture experiments, all humidifying devices were removed from the chamber.

Since there is no method devised at the present time for measuring the relative humidity at temperatures above 50° C., it was decided to measure the evaporating power of the atmosphere. This was done by the use of atmometers which consisted of two porous cups of the cylinder type connected to burettes on the outside of the chamber, all connections being made under water to eliminate air bubbles. These atmometers were carefully filled with boiled water, and placed on either side of the flat on which the plants were to be set. Notched corks were placed in the top of each burette to prevent evaporation at that point. After the chamber had been adjusted to a desired temperature, readings were made on the burettes at regular intervals. The results of these readings are shown in fig. 2.

At the high relative humidity, after the humidifying apparatus had been thoroughly adjusted, there was very little evaporation from the atmometers. Even at the higher temperatures, with no plants in the temperature chamber, there was less than 0.001 cc. evaporation per minute. These results give some indication of the conditions existing within the chamber, although it is not intended to imply that the plants lost water at exactly the same rate as the atmometers.

At temperatures below 50° C. an approximately saturated relative humidity for the higher moisture experiments and an average of 69 per cent for the lower moisture experiments were calculated from the readings of wet and dry bulb thermometers. When readings were made with the plants in the chamber, the relative humidity varied with the time of day, being as low as 55 per cent at night when transpiration was cut to a minimum and as high as 78 per cent at mid-day. Accordingly, most of the plants at the lower humidity were treated during the day. Since transpiration raised the relative humidity of the chamber, care was taken, when watering the plants in preparation for treatment, to keep the foliage dry and to allow the water to soak into the soil before placing them in the temperature chamber.

PROCEDURE

These experiments were made in order to determine the effects of humidity of super optimal and thermal death temperatures on

the cotton plant. They were suggested by the previous work of Berkley ('31) and the unpublished studies of Fung ('11). Cotton plants (variety Upland Big Ball) were grown in pots containing a mixture of sand and loam in a room of the greenhouse having a usual temperature of 25–30° C. Since individual variations made it necessary to use large numbers of plants of various ages, seeds were planted at frequent intervals from September, 1931, until the following September.

Two distinct series were run, one at a low, and the other at a high, relative humidity. The low-humidity experiments were made at temperatures between 42 and 84° C. inclusive, and those at the high humidity, between 40 and 65° C.

Plants of various ages (their ages being computed from the day the seeds were planted) were exposed to a particular temperature and humidity combination. After the chamber had been adjusted to the desired temperature, the plants were quickly passed through one of the glass doors, the slight drop in temperature occasioned by their entrance being quickly adjusted. They were removed after treatment to a compartment at room temperature which had a relative humidity similar to that of the treating chamber. The subsequent behavior of all plants treated was watched and the results noted. A minimum of 24 hours was allowed to elapse before the plants were pronounced dead. When the leaves and the growing tip of a plant were killed, it was called "dead" but was kept watered for some days to allow secondary growth to take place at the nodes in case the entire plant had not been killed. Such plants as did put forth secondary growth are listed in the tables. It will be noted that this was characteristic of the higher humidity experiments only.

SERIES I

Low humidity.—The plants treated at the lower relative humidity wilted to some extent before they were removed from the temperature chamber. When not too severely injured by excessive treatment, they revived and became turgid again immediately after removal. When treated for a longer time than was necessary to kill them, the wilted leaves and cotyledons temporarily regained their turgidity and to all appearances were

unharmful, but the petioles of the younger leaves, the stems just below the growing tips, and the hypocotyls of the seedlings were withered beyond recovery. After some hours, the time depending upon the intensity of the treatment, the injured portions of all plants, including those that temporarily recovered, dried up, only the cotyledons and leaves remaining green and turgid. These living organs, connected to the main stem of the plant only by dead tissue, remained in the green condition from 3 to 14 days before showing signs of death.

A microscopic study revealed no living tissue in the withered portions of the petioles and hypocotyls, but in the leaves and growing tips, which had recovered, the tissue was found to contain a number of mitotic figures. In the meristematic regions of the plants treated at times and temperatures just sufficient to kill them, the cell contents were disorganized.

Very few of the plants whose growing tips were killed put forth secondary growth at the nodes.

Tables I and II and fig. 3a give the detailed results of the lower humidity experiments. The thermal death point for the older plants treated at the lower relative humidity is in the neighborhood of 63° C., as shown by a sudden break in the line when the temperature is plotted against the time (fig. 3a). The time required to kill all of the plants at this temperature was about 7 minutes. The evaporation rate of the atmometer at 63° C. was 0.26 cc. per minute (fig. 2 and table II). If the plants were evaporating at a similar rate the cooling effect of the evaporation would be appreciable and would account for the greater length of time necessary to kill the plants at this temperature than at slightly higher temperatures.

The seedlings 10 days old or less had a slightly lower and more variable critical temperature (table I). The cotyledons and plumules showed about the same resistance as the older plants but the hypocotyls were permanently injured when treated for 8 minutes at 60° C. This injury to the hypocotyls ultimately caused the death of the entire plant.

In the older plants the petioles of the succulent young leaves were similarly affected but the plants were not killed unless the treatment was prolonged until the younger portion of the stem

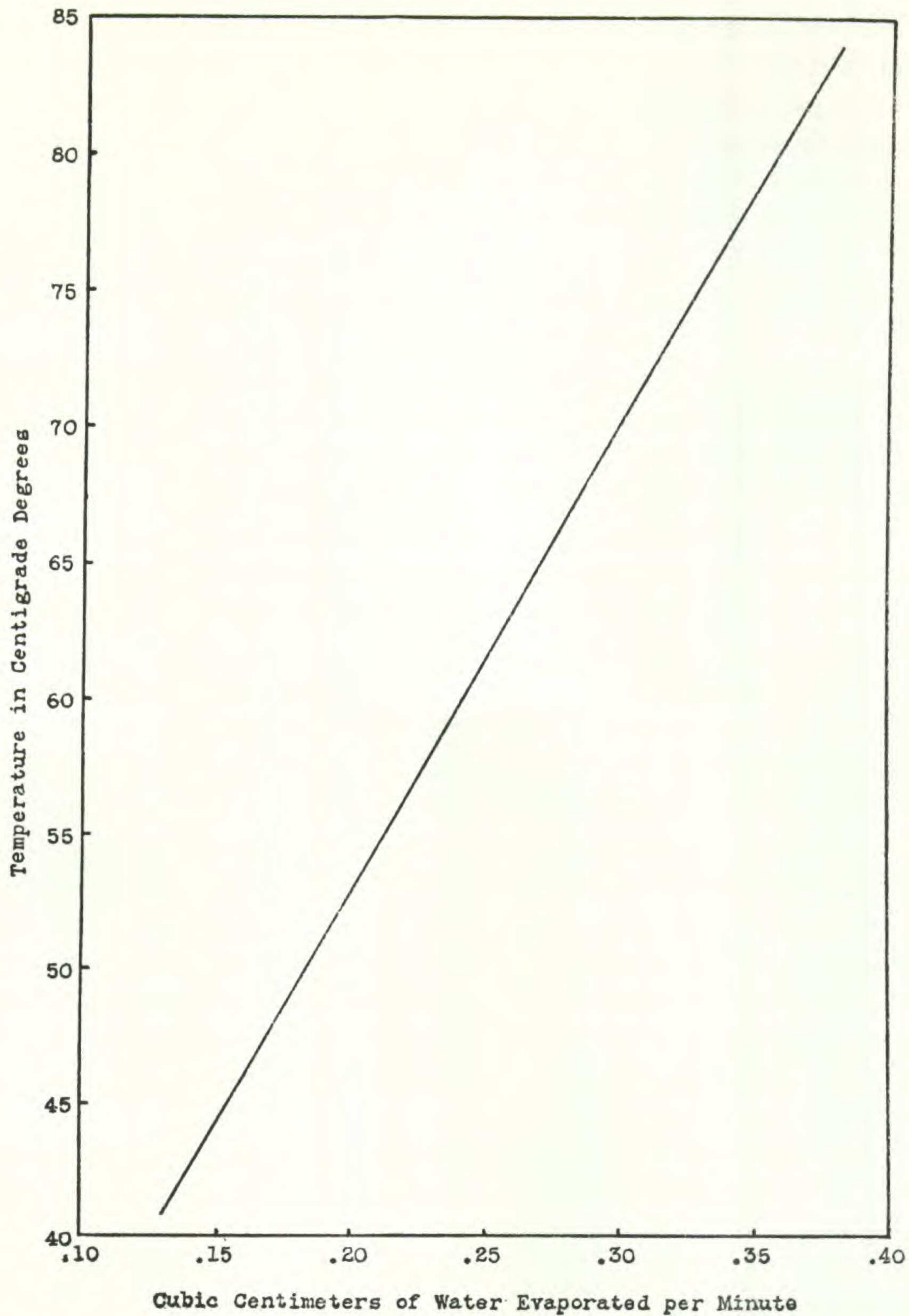


Fig. 2. The number of cubic centimeters of water evaporated per minute from an atometer, plotted against the temperature in Centigrade degrees. The determinations were made at 10° intervals. The figures in table II relating to the evaporation rate were taken from this graph.

and growing tip was injured. Therefore, the lower critical temperature of the seedlings can be attributed to the lack of resistance of the hypocotyl and not to the injuring of meristematic regions.

TABLE I
COTTON SEEDLINGS TREATED AT THE LOWER RELATIVE HUMIDITY

Temp. in degrees C.	Time	Age of plants (days)	Number of plants	Number living	Number dead	Evap. rate (cc. per min.)*
42	72 hr.	10	12	12		.14
45	45 hr.	24	9	6		.15
47	26 hr.	9	15	15		.16
52	3 hr.	10	12	12		.19
54	10 min.	8	32	32		.21
54	15 min.	8	6	1	5	
54	20 min.	8	10	4	6	
54	45 min.	8	20		20	
56	2½ min.	8	11	11		.22
56	5 min.	8	10	10		
56	7½ min.	8	10		10	
56	10 min.	8	8		8	
57	15 min.	8	40	1	39	.22
57	20 min.	10	20	8	12	
57	35 min.	8	66		66	
60	5 min.	10	15	15		.25
60	8 min.	8	55		55	
63	5 min.	8	65		65	.26
65	1 min.	10	17	17		.27
65	2½ min.	10	15	6	9	
65	5 min.	10	29		29	
67	1 min.	8	21	18	3	.28
68	1 min.	10	10	10		.29
68	2 min.	10	40		40	
69	1 min.	10	10	8	2	.29
69	2 min.	10	9		9	
70	1 min.	10	6	1	5	.30
70	2 min.	10	6	4	2	
70	4 min.	10	8		8	
74	1 min.	10	10	2	8	.32
74	1½ min.	10	21		21	
75	1 min.	10	4		4	.33
77	1 min.	10	11	1	10	.34
78	1 min.	10	6		6	.35
80	1 min.	10	11	3	8	.36

*This column shows the evaporation rate of water in cubic centimeters per minute from an atmometer, as described under "Apparatus," for the temperatures indicated.

In order to determine the cause of this differential resistance of the hypocotyls and cotyledons an experiment was made to find the amount of water lost during treatment and during various periods of time following treatment. This was done by deter-

TABLE II
COTTON PLANTS FROM 16 TO 180 DAYS OLD TREATED
AT THE LOWER HUMIDITY

Temp. in degrees C.	Time	Age of plants (days)	Number of plants	Number living	Number dead	Evap. rate (cc. per min.)*
42	24 hr.	25	6	6		.14
42	48 hr.	16	18	12	6	
42	72 hr.	53	6	4	2	
45	2 hr.	76	6	6		.15
47	18 hr.	77	36	36		.16
47	26 hr.	19	14	11	3	
47	26 hr.	76	6	6		
50	1½ hr.	35	12	10	2	.18
50	2¼ hr.	35	12	3	9	
52	3¾ hr.	55	48		48	.19
52	6 hr.	25	12	9	3	
53	1 hr.	78	28	28		.20
54	1 hr.	23	12	12		.21
55	30 min.	70	19	3	16	.21
55	30 min.	180	2	2		
55	45 min.	60	10	8	2	
55	1 hr.	24	18		18	
60	3 min.	32	11	4	7	.24
60	15 min.	70	25	5	20	
60	20 min.	70	27	2	25	
60	25 min.	30	55		55	
61	5 min.	32	16	4	12	.25
61	20 min.	32	128		128	
62	3 min.	32	7	3	4	.25
62	8 min.	32	10	2	8	
62	10 min.	32	50		50	
63	5 min.	72	14	2	12	.26
63	7 min.	72	18		18	
64	5 min.	32	82		82	.26
65	1 min.	40	8	8		.27
65	2 min.	40	8	4	4	
65	4 min.	40	113		113	
68	3 min.	40	73		73	.29
70	2 min.	70	16	2	14	.30
70	2½ min.	60	20		20	
70	3 min.	70	13		13	
74	1 min.	40	11	6	5	.32
74	1 min.	70	7	2	5	
74	1½ min.	40	11	1	10	
74	2 min.	40	10		10	
78	1 min.	40	11	2	9	.34
78	1 min.	70	4	3	1	
78	1 min.	120	3	2	1	
78	1½ min.	40	29		29	
80	1 min.	40	8	2	6	.36
80	1 min.	70	10	5	5	
80	1½ min.	60	32		32	
82	1 min.	70	8	2	6	.37
84	½ min.	70	7	5	2	.38
84	1 min.	70	11	1	10	
84	1¼ min.	60	10		10	

*This column shows the evaporation rate of water in cubic centimeters per minute from an atmometer, as described under "Apparatus," for the temperatures indicated.

mining the average percentage of moisture in the hypocotyls and cotyledons of treated and untreated seedlings (table III). The treated seedlings had been exposed for 15 minutes to a temperature of 60° C., a treatment known to leave the cotyledons of most of the seedlings in a living condition for a week or ten days.

In all, five sets were run: in one, which was used as a control, the plants were weighed before treatment, in one they were weighed immediately after treatment, in one, after 2 hours, in one, after 24 hours, and in another, 96 hours after treatment. Before weighing, the seedlings were cut about one-half inch above the surface of the soil and again immediately below the junction of the cotyledons and the hypocotyls. The hypocotyls and cotyledons were weighed separately, dried, and reweighed.

The results (table III) show that the hypocotyls and cotyledons had approximately the same water content before treatment, but the cotyledons lost considerably more during the treatment (3.3 per cent loss in the cotyledons as compared with 0.2 per cent in the hypocotyls). During the first 2 hours the cotyledons still showed a slightly more rapid rate of loss than the hypocotyls, but after 24 hours they had regained more than 3 per cent, while the hypocotyls still continued to lose. After 96 hours the hypocotyls were very much withered, having an average of only 78.3 per cent moisture, while the cotyledons still retained an average of 87.4 per cent and were more or less turgid.

TABLE III

LOSS OF MOISTURE IN SEEDLINGS DURING AND AFTER TREATMENT
AT 60° C. FOR 15 MINUTES AT LOW RELATIVE HUMIDITY

Number of plants		Per cent loss of moisture in hypocotyls	Per cent loss of moisture in cotyledons
400	Untreated	92.2	92.1
200	Treated & weighed immediately . .	92.0	88.8
200	Weighed 2 hours after treatment .	91.1	87.7
200	Weighed 24 hours after treatment	88.2	90.8
200	Weighed 96 hours after treatment	78.3	87.4

SERIES II

High humidity.—Under the high humidity conditions also the seedlings showed a somewhat lower and more variable critical temperature than did the older plants. When first removed from the temperature chamber, neither the seedlings nor the

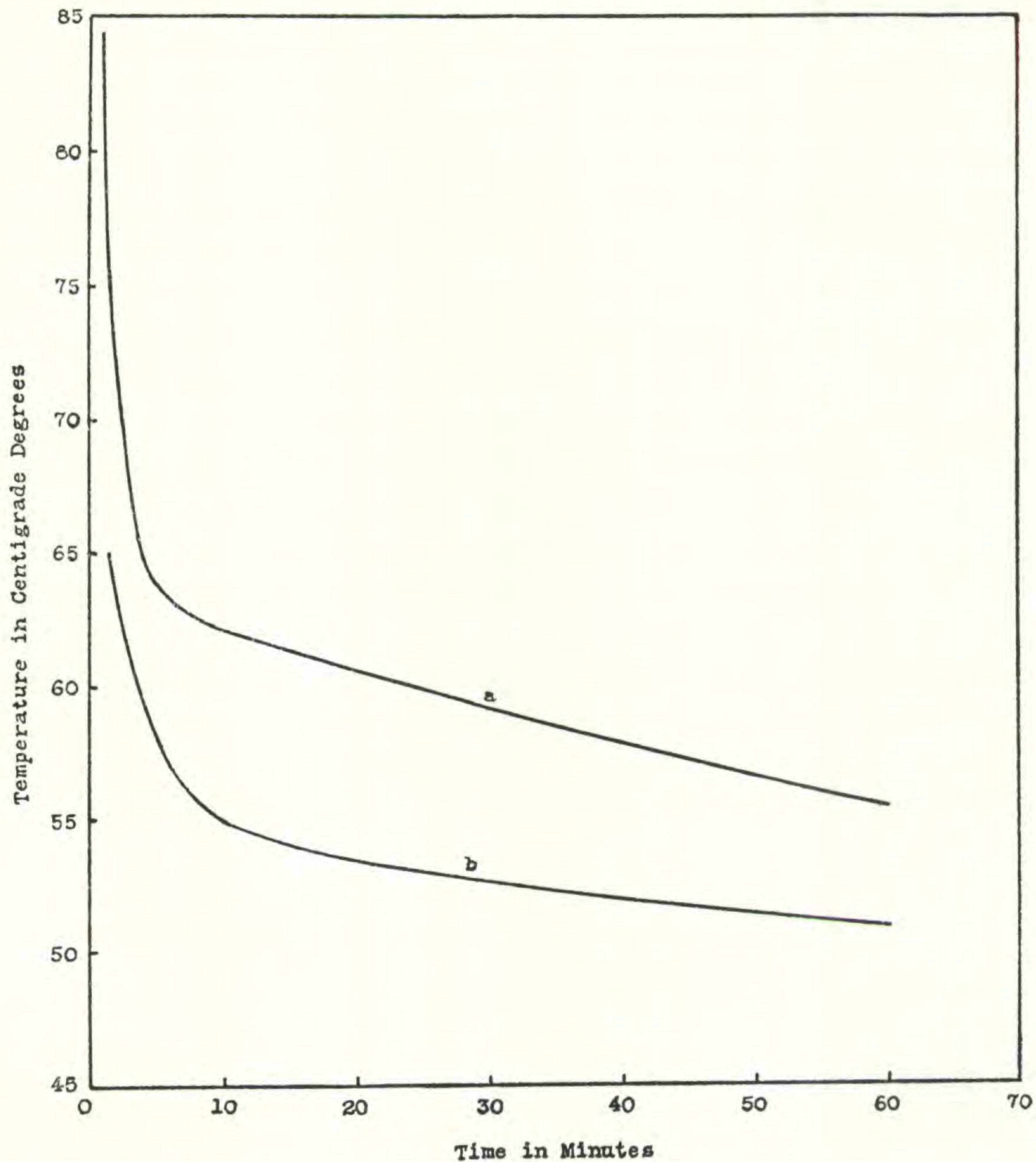


Fig. 3. The time in minutes necessary to kill all plants, plotted against the temperature in Centigrade degrees. The line (a) shows the results of the lower relative humidity experiments and (b) the results of the higher. Note the sharp break in (a). The lines in this graph are not drawn through any definite set of points but are plotted from tables II and V and are based upon the results obtained from the treatment of more than 4000 plants.

older plants showed any evidences of injury unless they had been left in the chamber for a period of time considerably longer than was necessary to kill them. In such a case, the young leaves and cotyledons appeared as if scalded. In the seedlings, injurious effects were later evidenced by the wilting or withering of the

TABLE IV
COTTON SEEDLINGS TREATED AT APPROXIMATELY
100 PER CENT RELATIVE HUMIDITY

Temp. in degrees C.	Time	Age of plants (days)	Number of plants	Number living	Number dead
41	24 hr.	12	22	4	18
41	48 hr.	13	22		22
42	9 hr.	10	6		6
45	2 hr.	10	6	6	
50	30 min.	14	12	2	10
50	45 min.	14	21	4	17
50	60 min.	14	24	8	16
52	10 min.	8	11	9	2
53	5 min.	15	6	6	
53	10 min.	9	6		6
54	3 min.	8	21	20	1
54	5 min.	8	15	6	9
55	5 min.	8	48	25	23
55	7 min.	8	23		23
57	2 min.	8	14	14	
57	3 min.	8	15	8	7
57	4 min.	8	15	2	13
58	2 min.	8	25	15	10
58	3 min.	8	16	3	13
58	5 min.	8	20		20
59	3 min.	8	23	1	22
59	5 min.	8	14		14
60	1 min.	8	8	8	
60	2 min.	8	22	12	10
60	2½ min.	8	40		40
61	2 min.	8	79		79
62	½ min.	8	9	9	
62	¾ min.	8	14		14
62	1 min.	8	37	3	34
62	2 min.	8	24		24
63	¾ min.	8	45		45
64	½ min.	8	54		54
65	½ min.	8	28		28

cotyledons, followed either by their abscission or by the drying up of the whole plant, the amount of injury and the time of its appearance depending upon the severity of the treatment. In older plants, injury appeared in the form of flaccidly wilted leaves and blackened growing tips which proved to be dead. In all cases, the growing tips were more resistant than the leaves, and even when the growing tips were killed secondary growth often appeared at the nodes. There was no withering of the hypocotyls and petioles (the cotyledons and leaves remaining green) as described for the lower humidity experiments.

Tables iv and v and fig. 3b give the detailed results of the higher humidity experiments. It will be noted from the tables and particularly from the figure that plants of any given age were much less resistant to high temperatures in the saturated atmosphere than at the lower humidity. The thermal death temperature evidently lies in the neighborhood of 55° C., if a definite point can be determined from this curve. This is 8° C. below that shown by the plants treated at the lower humidity.

TABLE V

COTTON PLANTS FROM 18 TO 120 DAYS OLD TREATED
AT APPROXIMATELY 100 PER CENT RELATIVE HUMIDITY

Temp. in degrees C.	Time	Age of plants (days)	Number of plants	Number living	Number dead	Secondary growth*
40	7 hr.	18	8	5	3	
40	10 hr.	19	30	7	23	
40	12 hr.	19	6		6	
41	24 hr.	50	30	24	6	
41	72 hr.	50	6	2	4	
42	9 hr.	35	26	26		
45	2 hr.	40	18	18		
46	2 hr.	18	12	12		
48	20 min.	26	6	6		
48	40 min.	26	6	5	1	
48	1 hr.	26	6	2	4	
48	1¼ hr.	26	6	3	3	
48	1¾ hr.	26	38		38	
50	30 min.	77	14	11	3	
50	45 min.	77	19	13	6	6
50	1 hr.	77	38	17	19	19
52	10 min.	72	11	11		
53	5 min.	26	6	1	5	
53	5 min.	36	6	3	3	
53	10 min.	35	102		102	
54	3 min.	72	11	11		
54	5 min.	70	4	2	2	2

TABLE V—Continued

Temp. in degrees C.	Time	Age of plants (days)	Number of plants	Number living	Number dead	Secondary growth*
54	10 min.	50	55		55	
55	3 min.	30	18	2	16	
55	5 min.	120	2	2		
55	7 min.	72	10	10		
55	9 min.	60	8		8	
56	5 min.	72	28	7	21	5
57	2 min.	73	2	1	1	1
57	4 min.	73	4		4	2
58	2 min.	73	7	3	4	
58	3 min.	70	4	3	1	1
58	4 min.	70	16	12	4	
58	5 min.	60	15		15	
59	2 min.	70	6	1	5	4
59	3 min.	70	5		5	
59	5 min.	73	11	1	10	
60	1 min.	73	1	1		
60	2½ min.	120	3	3		
60	3 min.	73	15	6	9	2
60	4 min.	60	9		9	
61	1½ min.	75	3	1	2	
61	2 min.	73	10		10	
62	½ min.	73	5	5		
62	¾ min.	73	8	5	3	2
62	1 min.	73	26	2	24	3
62	2 min.	72	8	5	3	2
62	2½ min.	60	10		10	
62	3 min.	72	11		11	3
63	¾ min.	73	6		6	4
63	1 min.	73	3	1	2	
63	1¼ min.	73	4		4	1
64	½ min.	73	9		9	4
64	1 min.	73	12		12	3
65	½ min.	73	2	2		
65	¾ min.	73	6	1	5	4
65	1 min.	73	5	2	3	
65	1¼ min.	60	10		10	

*This column shows the number of plants that put forth secondary growth at the nodes after the growing tips had been killed.

DISCUSSION

When the data of the two sets of plants were compared, it was found that the cotton plant was much more resistant to high temperatures under the lower-humidity conditions. Furthermore, the nature of the injury and the parts of the plant first to be affected were entirely different in the two sets. At the lower relative humidity, the petioles, young stems, and the hypocotyls were the first to be killed, whereas at the higher relative humidity they were the last to be affected, the leaves and cotyledons

dying first. The seedlings treated at the lower relative humidity were invariably killed by injury to the hypocotyls.

Experiments showed that the cotyledons of the seedlings treated at the lower relative humidity lost moisture at a much greater rate than the hypocotyls during the treatment. Immediately following their removal from the temperature chamber the hypocotyls lost moisture rapidly while the cotyledons regained a large percentage of that lost during treatment. This would indicate that the evaporation of the water immediately utilized enough of the heat energy around the leaves and cotyledons to protect them until the other portions became injured and even killed. This assumption is further substantiated by the fact that all plants were so readily killed in the saturated atmosphere where transpiration was reduced to a minimum.

These experiments tend to prove the statement first made by Sachs ('64) and later by Ewart ('03) and Baker ('29) that reduced transpiration allows a more rapid concentration of heat in the plant. This phenomenon has not been emphasized by sufficient experimental data to show its true significance, and apparently not in any case has it been previously demonstrated under the conditions of thermal death temperatures. Clum ('26) claimed that this cooling effect was greatly over-estimated, but his experiments on thermal death temperatures were not sufficiently well controlled to justify his statement.

In the light of the facts shown by the present experiments it is evident that the thermal death point has not as yet been clearly defined. It will be necessary to limit the term to the death of the protoplasm alone and not to the entire plant. The death of the plant might be caused by the injuring or killing of some portion or organ which would prevent normal functioning, as shown by the seedlings treated at the lower relative humidity.

Local injury which is not outwardly evidenced may occur at temperatures below that designated as the thermal death temperature. Litardière ('25), working with onion root tips, found that the nuclei were affected at 48° C. after 24 hours and that the injury increased with higher temperatures even for shorter periods of time. Yamaha ('27) gave 38° C. as a critical temperature for the root tips of the bean, and Milovidov ('32), working

with a number of different plants, found that temperatures around 45-47° C. were injurious to the plants studied if the treatment were prolonged. The cursory microscopic studies made on the cotton plants in the present work showed that temperatures of 55° C. and above, even for very short periods of time, were injurious to the protoplasm, causing plasmolysis and deformation of the nuclei. Since these were not uniformly distributed in the plant it would be difficult to estimate their ultimate effects.

Blackman's idea of the "extinction temperature"¹ ('05) necessitates the setting of an arbitrary time limit which, in the opinion of the writers, is not justified since the time required for the protoplasm itself to assume the thermal death temperature would vary with atmospheric conditions under which the plants were treated.

At present no entirely satisfactory definition of the thermal death point can be given, but the following may serve until more data is available: *The thermal death point is that temperature which, at a given relative humidity, will kill the protoplasm immediately upon its assumption.* Were it not for the indication that humidity has an effect in addition to preventing cooling caused by the retardation of the transpiration rate, it would be possible to eliminate from the definition the qualifying phrase dealing with humidity. If the humidity had no other effects it would merely vary the time required for the plant cells to assume the temperature necessary to kill the protoplasm. This does not appear to be the fact, however, since there is so much difference between the low- and high-humidity experiments. It is likely that the extremely high relative humidity has an additional effect, that of smothering the plants. When plants from an atmospheric temperature of 25-30° C. were abruptly transferred into a temperature of 50-60° C. and a practically saturated atmosphere, condensation immediately took place on the surface of the leaves and stems. By reducing the transpiration stream

¹ " . . . we ought to find a temperature at which the earliest estimation that could be actually made would give no measurable assimilation. The lowest temperature to give this result might be called the 'extinction temperature' . . . (say in 100 seconds, for the accepted specific extinction temperature would of course have to be arbitrarily defined in time units)."

and the usual exchange of gases, this film of water would naturally limit the oxygen supply of the plant. Since plants in the higher temperature would demand more oxygen than could be obtained, it is possible that the protoplasm was killed by a combination of factors. The evaluation of the extent and full importance of this smothering effect will necessitate further experimentation.

In regard to the time limit as a criterion of the thermal death point, we must consider the factors influencing the rapid change of temperature of the plants from that of the atmosphere in which they were grown to that of the treating chamber. Some of these factors are transpiration in the plant, condensation of moisture on its surface, and temperature gradients immediately around the plants. Temperature gradients can be prevented by keeping the air thoroughly mixed, but it is very difficult to determine the exact relations of transpiration or condensation and the change in temperature of the protoplasm.

An examination of fig. 3a shows a sharp break in the line between 60 and 65° C., which marks the critical temperature for the cotton plant at the low relative humidity. A thermal death temperature of 63° C. may then be chosen, but that is the temperature of the atmosphere. It will be noted that it took from 5 to 7 minutes to kill the plants at 63° C. and progressively shorter intervals of time as the temperature increased, which would indicate that the protoplasm died immediately upon assuming the temperature of the atmosphere. The time lag, caused primarily by the cooling effect of transpiration, makes it necessary either to determine the exact temperature of the protoplasm or, more simply, to plot the temperature against the time and determine from the graph thus obtained the approximate thermal death point.

It is true that prolonged temperatures below 63° C. will kill the plants, but it is also evident from the slope of the upper portion of the line that it did not take, for example, 25 minutes for the protoplasm to assume a temperature of 60° C. when treated at this temperature. Then they were killed gradually, and in such a case the reactions set up by the high temperatures may have led to the death of the protoplasm.

It may be concluded that no definite temperature can be set

as the thermal death point of the cotton plant without stating first the atmospheric conditions under which the plants were treated and giving the ages of the plants. Transpiration plays a definite role in the cooling of the leaves and cotyledons under low relative humidity conditions.

SUMMARY

1. Two series of cotton plants 5 to 180 days old were exposed to super optimal and thermal death temperatures for periods of time ranging from $\frac{1}{2}$ minute to 72 hours. In Series I the plants were exposed to temperatures of 42-84° C. at an average relative humidity of 69 per cent at temperatures below 50° C. The evaporation rate was substituted for relative humidity in this series. In series II the plants were exposed to temperatures of 40-65° C. at an approximately saturated atmosphere.

2. Seedlings were less resistant to high temperatures at any given relative humidity than older plants.

3. The plants were less resistant to high temperatures at the higher relative humidity.

4. At the higher relative humidity, the leaves and cotyledons were the first parts of the plant to be affected.

5. At the lower relative humidity, the hypocotyls of the seedlings and the petioles and young stems of the older plants were the first to be affected.

6. During treatment at the lower humidity, the cotyledons lost water rapidly whereas the hypocotyls lost very little. After removal from the treating chamber the cotyledons regained a large percentage of the water lost and became turgid again, whereas the hypocotyls continued to lose moisture until they were completely withered.

7. The more rapid rate of transpiration in the cotyledons apparently utilized sufficient heat energy to protect them from being noticeably injured until the hypocotyls were killed. These data tend to substantiate the theory that the cooling effect of transpiration is of great value to plants in preventing an accumulation of heat energy.

8. The saturated atmosphere of Series II appeared to have an additional effect, that of smothering the plants. This was

apparently due to the retardation of the gas exchange caused by the condensation of moisture on the surface of the plants, thus reducing the oxygen supply.

9. Due to the two-fold effects of humidity the following is given as a tentative definition of the thermal death point: The thermal death point is that temperature which, at a given relative humidity, will kill the protoplasm immediately upon its assumption.

10. No definite temperature can be given as the thermal death point of the cotton plant without stating the humidity of the atmosphere and the age of the plant.

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